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ENZYMATIC DEGRADATION OF HD

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An enzyme, derived from a Rhodococcus bacterium, was found to have catalytic activity with HD as the substrate. The enzyme has previously been characterized as a hydrolytic dehalogenase and apparently catalyzes the hydrolysis reaction of HD, because chloride is released stoichiometrically. The enzyme is precipitated between 60 and 100% ammonium sulfate saturation, binds to Q Sepharose and elutes at an ammonium sulfate concentration of about 0.1%. The enzyme exhibits saturation kinetics with HD as its substrate. The Km is about 15 mM HD and the Vmax occurs at about 25 mM. It exhibits fairly conventional temperature and pH effects with a temperature optimum of about 35 °C and a pH optimum of about 7.0.

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PREFACE

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Enzymatic Degradation of HD

1. INTRODUCTION

One potentially promising approach to the decontamination of toxic chemicals is the use of catalytic enzymes in an aqueous matrix. Enzymes often have extremely high catalytic rates and are very stable under dehydrated storage conditions. These characteristics are consistent with their use in a concentratable, catalytic, aqueous decontaminant. In the particular case of decontamination of chemical warfare agents, the reduction in logistical burden and decreased environmental hazard from the use of such a decontaminant could offer significant advantages.

The enzymes organophoshorus acid anhydrolase (OPAA), from *Alteromonas sp.* JD6.5 and organophosphorus hydrolase (OPH) have been shown to catalyze the hydrolysis of a number of toxic organophosphorus compounds including several G-type chemical nerve agents.[1-12] The OPH also has catalytic activity against V-type nerve agents.[13] These enzymes have been cloned into *Escherichia coli* and can be produced in significant quantities. The OPH enzymatic activity for specific substrates has also been increased by genetic manipulation of the clone.[14] The catalytic activity of these enzymes against nerve agents also suggests their potential use for decontamination and/or *in vivo* prophylaxis.

To date, there have been no reports of the enzymatic degradation of HD (sulfur mustard, 2,2'-dichlorodiethyl sulfide). The lack of an HD enzyme has thus far prevented the formulation of a broadrange enzyme-based catalytic decontaminant for G, V, and H-type chemical agents. Nevertheless, a number of enzymes have been reported degrade chlorinated compounds with some structural similarity to HD. For instance, the hydrolytic dehalogenase enzyme from *Rhodococcus* strain GJ70 (ref) has catalytic activity against 1,6-dichlorohexane and 1,5-dichloropentane.

Hydrolytic dehalogenase enzymes are particularly interesting for decontamination because they do not require cofactors and because the hydrolysis reaction yields thiodiglycol (TDG) from HD. The difference in toxicity between HD and TDG is a factor of 4,200 to 5,700 (the oral LD_{50} of HD is 0.7 mg/kg[15] whereas that of TDG is 3000 – 4000 mg/kg[16]). This reduction in toxicity offers the potential to seriously reduce the damage caused by HD if it can be decontaminated quickly enough.

Most hydrolytic dehalogenase enzymes described in the literature are found *Rhodococcus* bacteria.

2. MATERIALS AND METHODS

2.1 Bacteria.

Strain BL21 was a kind gift from EIKOS. It was grown in Luria broth augmented with 100 µg/mL ampicillin. Cells were harvested at an OD_{600} of approximately 1.0 following 3 hr growth post-isopropylthiogalactoside induction at an OD_{600} of approximately 0.1.

2.2 Chemicals.

The HD was stabilized with tributylamine and was approximately 90% pure.

2.3 Enzyme Assays.

Enzyme assays were conducted with a chloride electrode attached to a Fisher Accumet 925 m. Reactions were conducted in a temperature-controlled vessel in a total volume of 5 mL. Buffering was provided by a 50 mM solution of MOPS at pH 7.2. Data logging was automated through an RS-232 connection to a computer.

3. RESULTS AND DISCUSSION

3.1 Initial Partial Enzyme Purification.

3.1.1 Ammonium Sulfate Precipitation.

Ammonium sulfate was added to 20, 40, 60, 80, and 100% of saturation. The precipitated protein was centrifuged at 10,000 x g for 30 min, and the activity of the respective supernatant solutions was determined. Activity of the supernatant solutions is shown in Figure 1.

3.1.2. Q Sepharose Chromatography.

The pellets from the 60, 80, and100% precipitations were combined, resuspended in 10 mM Tris-SO_4 pH 7.5 1 mM ethylenediaminetetracetic acid 1 mM B-mercaptoethanol (TEM) buffer (4 mL/g of cells recovered) and dialyzed overnight against TEM with repeated buffer changes. The dialyzed material was then loaded onto a 40 mL Q Sepharose column and eluted with a 0 – 1 M ammonium sulfate gradient. The HD activity and OD_{280} (estimated protein concentration) are shown in Figure 2. Essentially all the HD activity was found coincident with the largest protein peak. Q Sepharose Fraction 3, although not pure by polyacrylamide gel electrophoresis, was used for initial determinations of assay conditions that follow below.

3.2 Determination of Optimal Activity Assay Conditions

3.2.1 Substrate Concentration.

The activity of Fraction 3 was tested with various HD concentrations ranging from 3.27 to 51.11 mM to determine the approximate Km. The substrate vs. activity curve is shown in Figure 3. The Km was approximately 15 mM HD, as calculated with EZFIT software. The Vmax occurred at approximately 25 mM HD, so this concentration was used for other tests to determine the optimal activity conditions.

3.2.2 Temperature Effects.

Using the optimal substrate concentration of 25 mM, assays were conducted at varying temperatures to determine temperature effects on the enzyme. Because the freezing point of HD is about 15 °C, the lowest temperature tried was 20 °C.

3.2.3 pH Effects.

Using the optimal substrate concentration of 25 mM and the optimal temperature of 35 °C, assays were conducted at varying pH to determine the effects on the enzyme activity. The MOPS buffering range limited the testing range between approximately pH 6.5 and pH 8.5

4. CONCLUSIONS

An enzyme, derived from a *Rhodococcus* bacterium, was found to have catalytic activity with HD as the substrate. The enzyme has previously been characterized as a hydrolytic dehalogenase and apparently catalyzes the hydrolysis reaction of HD, because chloride is released stoichiometrically. To our knowledge, it is the first enzyme described to catalyze the hydrolysis of HD.

The enzyme is precipitated between 60 and 100% ammonium sulfate saturation.

The enzyme binds to Q Sepharose and elutes at an ammonium sulfate concentration of about 0.1%.

The enzyme exhibits saturation kinetics with HD as its substrate. The Km is about 15 mM HD and the Vmax occurs at about 25 mM.

The enzyme exhibits fairly conventional temperature and pH effects with a temperature optimum of about 35 °C and a pH optimum of about 7.0.

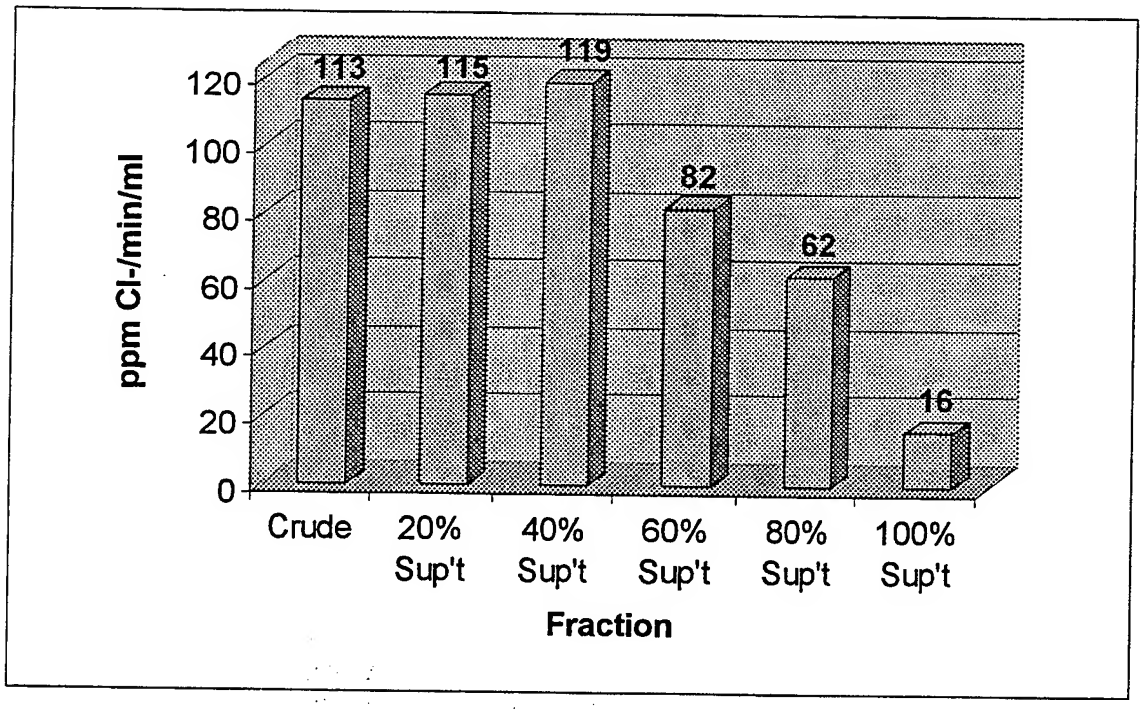

Figure 1. Activity of the Supernatants of Respective Ammonium Sulfate Fractions

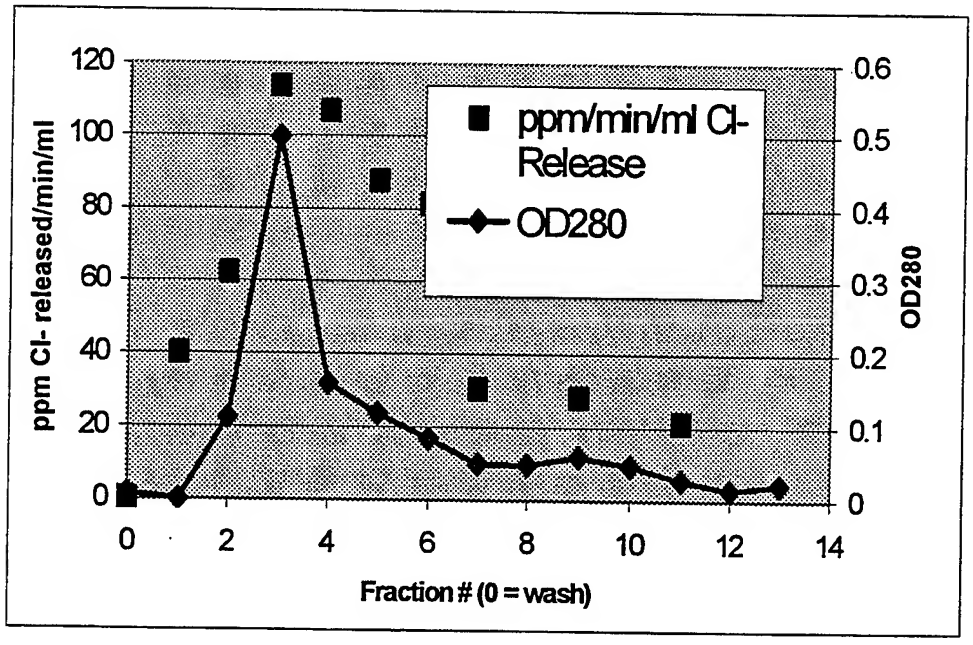

Figure 2. HD Activity and OD_{280} Profile of Q Sepharose Chromatogram With 0 – 1 M Ammonium Sulfate Gradient

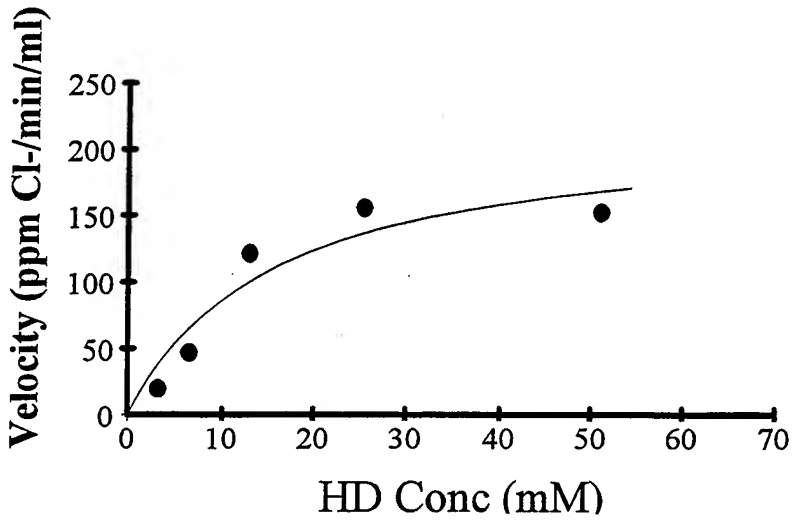

Figure 3. Substrate vs. Velocity Curve of Q Sepharose Fraction 3

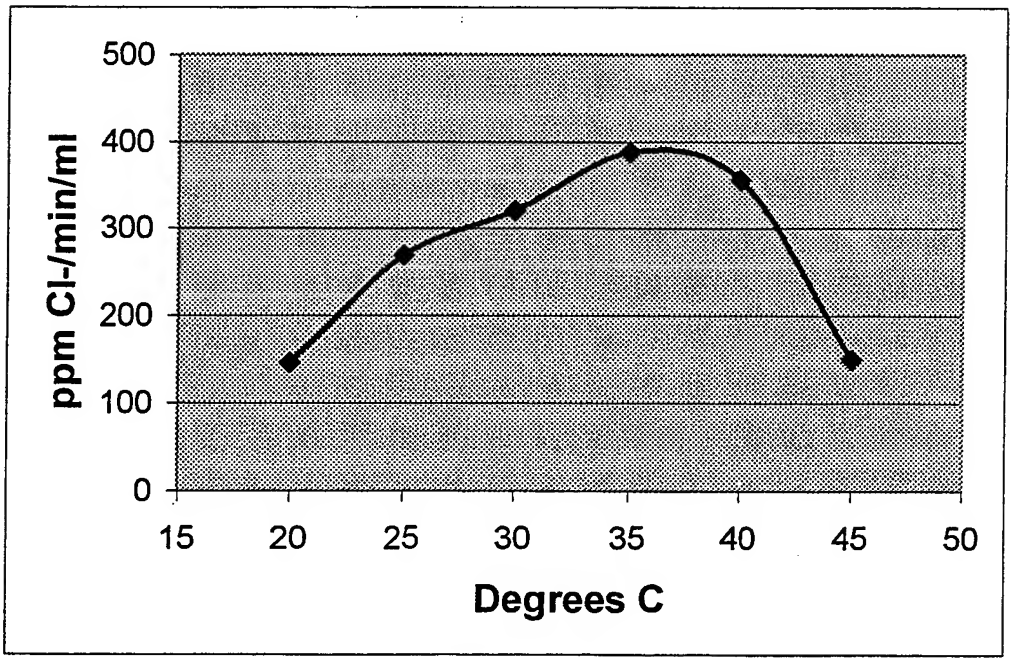

Figure 4. Temperature Profile of HD Enzyme Activity. All assays were performed at 25 mM HD in 50 mM MOPS buffer pH 7.2.

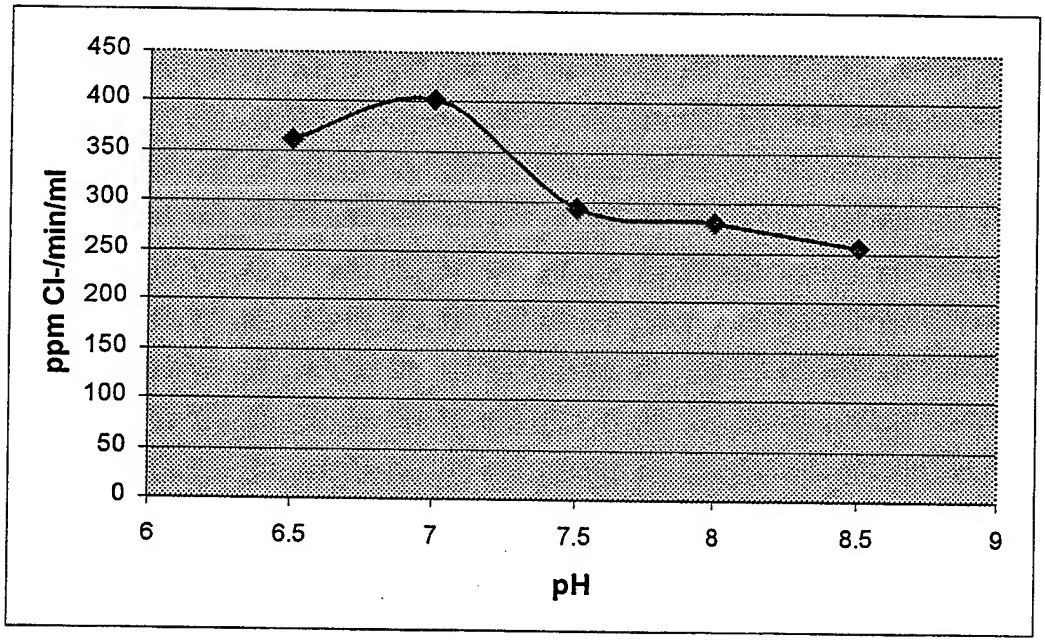

Figure 5. pH Profile of Enzyme Activity Against HD, Measured Within the Approximate Effective Buffering Range of MOPS Buffer

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